PB86-203734

Pesticide Assessment Guidelines, Subdivision O Addendum. Residue Chemistry Data Requirements for Analytical Methods in 40 CFR Part 158.125 - Multiresidue Protocols

(U.S.) Environmental Protection Agency
Arlington, VA

May 86

U.S. Department of Commerce
National Technical Information Service
NTIS

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	3 PB86-203734

4. Title and Subtitle
Hazard Evaluation Division
Pesticide Assessment Guidelines
Residue Chemistry

5. Report Date May 1986

6.

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8. Performing Organization Rept. No. EPA-540/9-86-148

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10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.
(C)
(G)

12. Sponsoring Organization Name and Address
Same as #9

13. Type of Report & Period Covered

14.

15. Supplementary Notes Supplement to Pesticide Assessment Guidelines Subdivision O -ADDENDUM
Residue Chemistry: Residue Analytical Methods, 40 CFR Part 158.125, Data Requirements for Pesticide Registration

16. Abstract (Limit: 200 words)

This supplement on Residue Analytical Methods is primarily for the regulated industry in implementing the Residue Chemistry Data Requirements, 40 CFR §158.125(b)(15), such that uniform FDA/USDA multiresidue methodology testing is now required for all pesticides. For this purpose, the attached Protocols I, II, III, and IV are added as required schemes for testing of each pesticide. Use of these testing schemes Protocols I-IV, may indicate multiresidue methods are more suitable for the identificattion and determination of pesticide residues than those methods designated for the individual pesticides found in the Pesticide Analytical Manual Volume II (PAM-II). The FDA Protocols I-IV have been added as an Appendix to the Pesticide Assessment Guidelines, Subdivision O-Residue Chemistry.

The data developed under these Protocols will be published as entries in appropriate tables in the Pesticide Analytical Manual, Volume I. The data are for the use of any agency responsible for enforcing tolerances or monitoring residues and thus are not to be claimed as Confidential Business Information (CBI).

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
Unclassified and Freely Available	Unclassified	17
	20. Security Class (This Page) Unclassified	22. Price

May 1986

HAZARD EVALUATION DIVISION

PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION O - ADDENDUM

RESIDUE CHEMISTRY DATA REQUIREMENTS FOR ANALYTICAL METHODS IN 40 CFR PART 158.125 - MULTIRESIDUE PROTOCOLS

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Multiresidue Method Testing

Analytical methods capable of determining many pesticide residues in a single analysis have long provided the basis for the U.S. Food and Drug Administration's programs to determine residue levels in the U.S. food supply. Through the years, as the several multiresidue methods have been added to FDA's programs, a concomitant effort has been made to test as many potential residues as possible through these methods. All the data collected from tests run according to specified protocols have been compiled and distributed.

Specific directions for each multiresidue method used by FDA are published in the agency's Pesticide Analytical Manual Vol. I (PAM I) as stated in 40 CFR 180.6(d) and 180.101(c). Compilation of data on the analytical behavior of pesticides and related chemicals are also published in PAM I. The data compiled in this way include: relative retention times of the compounds on a variety of gas-liquid chromatographic (GLC) columns; responses of various GLC detectors to the compounds; recovery of the compound through complete methods, and sometimes through important steps within the methods. The large amount of effort spent on the testing of multiresidue methods and compilation of results is justified by the advantages such compilations offer the analytical chemist. When analytical behavior data for numerous compounds through the method in use is known, the analyst is better equipped to recognize the residues that are present in a sample of unknown treatment history. In situations where the likelihood of some particular residue is known, the data lists for several methods can be consulted to help choose which method should be used.

Regulatory agencies which must assess the incidence of residue occurrence are also assisted by data compilations. The absence of many chemicals from the sample can be ascertained when it is known that the compounds could have been detected had they been present.

It has been found advisable to define protocols to follow in developing data on multiresidue method behavior. In order to compile data into usable formats, it is imperative that all contributing laboratories perform the tests in the same way. The goal in these method-testing exercises is not to find the optimum conditions for the one compound currently being tested, but to be able to describe how the compound will behave when determined by the precisely-defined method.

To this end, four protocols follow. Each references the PAM I method(s) involved, the types of compounds to which it applies, and the PAM I table(s) in which previously-collected data are published. The data development section of each

protocol provides the directions for setting instrument parameters and for testing the compounds through the parts of the method.

Follow the steps of these protocols, in the order written, to develop data which can be compiled with that from other laboratories. These data will be included in PAM I tables and distributed to the many laboratories around the world which use these methods.

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Food and Drug Administration
September 1984

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Protocol I

Background

Methods: PAM I 211.1, 212.1, 252

Compound Type: Generally, non-polar compounds capable of detection by electron capture or phosphorus-selective GLC detectors. A wide variety of compounds are recovered through these methods; do not assume too readily that the compound is too polar to be recovered. [Note that the protocol is set up to test for recovery through the Florisil cleanup column first, so that time will not be wasted testing through the complete method compounds which are not eluted from Florisil.]

PAM I Tables: Appendix (all data)

Table 201-A (recoveries from Florisil and through complete methods)

Tables 331-A, 331-F, 331-G, 334-A (GLC data)

Data Development

I. Instrumentation

A. GC column

1. Required column: 5% OV-101 or equivalent
2. Additional columns of interest:
   a. 3% OV-225
   b. 2% DEGS (ANALABS)
   c. 3% OV-17

B. GC detectors

1. Electron Capture (^{63}Ni constant current)
2. Hall 700 A (halogen)
3. Flame Photometric Detector (phosphorus)

C. GC parameters

1. Adjust column temperatures, 5% OV-101, so that relative retention to chlorpyrifos (RRc) of p,p'-DDT is 3.10 $\pm$ 0.03 (or RRc of ethion is 2.56 $\pm$ 0.03) (see PAM I 331A for RRc data).

   For other columns of interest, see the following tables for operating conditions and RRcs:

a. Table 331-F - OV-225 column
b. Table 334-A - DEGS column
c. Table 331-G - OV-17 column

2. Detector sensitivity: Select electrometer attenuation to produce 50% full scale deflection (FSD) for injection of 1.5 ng chlorpyrifos.

See the following PAM I sections for parameters for the various detectors:

a. Section 311.4 - Electron capture
b. Section 315 - Hall 700 A (halogen)
c. Section 314 and Table 331-G - Flame photometric

II. GC analytical behavior of new compound

A. Dissolve reference standard material in pesticide-grade solvent (iso-octane preferred) to prepare stock standard solution.

B. Inject aliquots containing up to 1000 ng into GC system to determine the amount of standard required to cause 50% FSD at the prescribed parameters.

C. Calculate RRc of new compound for all GC column/detector systems used.

III. Recovery of new compound through cleanup column

A. Determine recovery through Florisil column, PAM I 211.14d and 252.12b. Use only Florisil which has been shown to permit elution of heptachlor epoxide in 6% EE/PE or eluate 1 and endrin in 15% EE/PE or eluate 2. Add 10-100 ug of analyte in 1-10 ml hexane solution to each Florisil column experiment in duplicate. If recoveries are less than 30%, report recoveries through Florisil and terminate work on this compound through this method.

B. If new compound elutes in 6% EE/PE or eluate 1, rerun Florisil column experiment in the following manner.

(1) Elute Florisil column with 250 mL petroleum ether.
(2) Proceed with 6% EE/PE or eluate 1.

Report percent recovery and eluate(s) which contains analyte.

IV. Recovery of new compound through complete methods

A. Select one representative fatty and one nonfatty food

sample. Analyze food first to assure that there are no residues in the sample which might interfere with the fortification test. Simultaneously, analyze a reagent blank for further information on the source of possible interferences.

Fortify 100 g samples in duplicate at approximately 0.05 ppm. Analyze duplicate fortified samples as described in PAM I 211.1 and 212.1 for fatty foods and nonfatty foods, respectively.

B. Fortify 100 g samples in duplicate at the tolerance level or, if no tolerance exists, at approximately 0.5 ppm. Analyze duplicate fortfied samples.

C. If the compound is recovered in experiments IV. A and B above, and if it was recovered in III. A using the Florisil elution system PAM I 252, then repeat IV A and B using the PAM I 252 elution system.

V. Report all results in a format which parallels this protocol. Include results of checks made on instrument parameters, as described in I.C.

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September 1984

Protocol II

Background

Method: PAM I 232.3

Compound Type: Organophosphorus pesticides and their polar metabolites. Also certain organonitrogen compounds, if the GLC detector used responds to nitrogen.

PAM I Tables: Appendix (all data)

Table 201-H (recoveries through complete method)

Table 334-A (DEGS column RRcs)

Tables 331-F, 331-G (GLC data)

Table 333-A (relative retention times on DC-200 column, equivalent to OV-101; detector response data for alkali flame (KCl thermionic) detector, rather than FPD.)

Data Development

I. Instrumentation

A. GC column

1. Required column: 4 ft x 2 mm (i.d.) 2% DEGS - (precoated packing, available from Analabs, Inc., Cat. No. GCM-035)
2. Additional columns of interest:
   a. 3% OV-225
   b. 3% OV-17
   c. 5% OV-101

B. GC detectors

1. Flame photometric detector, phosphorus mode (FPD-P). This is the preferred detector for the determination of new compound data.

2. Alkali flame detector or N/P detector. (These detectors should be used only if FPD-P detector is not available.)

C. GC parameters

1. Operate DEGS column at 180°C; adjust temperature as needed to produce RRcs for parathion and monocrotophos

as reported in PAM I Table 334-A.

For other columns of interest, see the following tables for operating conditions and RRcs:

a. Table 331-F - OV-225 column
b. Table 331-G - OV-17 column
c. Table 331-A - OV-101 column

2. Detector sensitivity: Select electrometer attenuation so that injection of 1.5 ng chlorpyrifos produces 50% full scale deflection (FSD) on recorder or printer/plotter.

See the following PAM I sections for parameters for the various detectors:

a. Table 331-G - FPD
b. Section 313 - KC1TD
c. Section 316 - N/P

II. GC analytical behavior behavior of new compound

A. Dissolve reference standard material in pesticide-grade acetone to prepare stock solutions and all subsequent dilutions. Also prepare acetone solutions of chlorpyrifos, parathion, and monocrotophos for accompanying work.

B. Determine amount (ng) of new compound which produces 50% FSD.

C. Calculate RRc of new compound for all GC columns/detector systems used.

III. Recovery of new compound through cleanup column

Determine recovery of compound, in duplicate, through PAM I 232.34 column chromatography. Add 5-50 ug of compound to cleanup column and elute as instructed. If recovery is less than 30%, do not proceed to test compound through entire method. Report recoveries through clean-up column alone.

IV. Recovery of new compound through complete method

A. Select a representative nonfatty food sample. Analyze food by this method to assure that there are no residues in the sample which might interfere with the fortification test. Simultaneously, analyze a reagent blank for further information on the source of possible interferences.

Fortify 100 g samples, in duplicate at 0.05-0.10 ppm. Analyze the duplicate fortified samples as described in PAM I 232.3.

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B. Fortify 100 g samples in duplicate at the tolerance level or, if no tolerance exists, at 5 x level of A above. Analyze duplicate fortified samples.

V. Report all results in a format which parallels this protocol. Include results of checks made on instrument parameters, as described in item I.C.

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Protocol III

Background

Method: PAM I 232.4

Compound types: Applicable to all nonionic types of pesticides; detection of particular compounds is dependent on availability of the various element-selective detectors which can be used.

PAM I Tables: Appendix (GLC data)

Table 201-I (recoveries through the complete method)

Tables 331-A, 331-F, 331-G, 334-A (GLC data)

Data Development

I. Instrumentation

A. GC columns

1. Organochlorine compounds

a. Required column: 5% OV-101 or equivalent
b. Additional columns of interest:
(1) 3% OV-17
(2) 3% OV-225

2. Organophosphorus/organonitrogen compounds

a. Required column: 4' x 2 mm (i.d.) 2% DEGS (precoated packing, available from Analabs, Inc. Cat. No. GCM-035)
b. Additional columns of interest:
(1) 3% OV-225
(2) Ultrabond 20 SE (precoated packing available from Ultra Scientific Cat. No. RGC-023A)
(3) 3% OV-17
(4) 5% OV-101

B. GC detectors

1. Organochlorine compounds

a. Preferred detector: Hall 700 A (halogen)

b. Other detector: electron capture (^{63}Ni constant current). If an electron capture detector must be used, the analytical methodology must include Florisil clean-up (PAM I 212.2) prior to GC determination. (PAM I 311.4).

2. Organophosphorus compounds

a. Preferred detector: flame photometic detector phosphorus mode (FPD-P).

b. Other detectors: alkali flame detectors (KClTD or N/P).

3. Organonitrogen compounds

a. Alkali flame (KClTD or N/P)

b. Hall 700 A (nitrogen)

C. GC parameters

1. Column temperatures

a. OV-101: operate at 200°C. Adjust temperature, if necessary, to give RRc for p,p'-DDT = 3.10 ± 0.03. See PAM I 331-A for additional RRc.

b. DEGS: operate at 180°C. Adjust temperature, if necessary, to give RRc for parathion and monocrotophos as noted in PAM I, Table 334-A.

c. OV-17. See Table 331-G for conditions.

d. OV-225. See Table 331-F for conditions. Note that this column cannot be used with electrolytic conductivity detectors or N-selective detectors.

2. Detector sensitivity: Select electrometer attenuation so that injection of 1.5 ng chlorpyrifos produces 50% full scale deflection (FSD) on recorder or printer/plotter.

See the following PAM I sections for parameters for the various detectors:

a. Section 315 - Hall 700 A
b. Tables 334-A, 331-G - FPD-P
c. Section 311.4 - ^{63}Ni electron capture
d. Section 313 - KClTD
e. Section 316 - N/P

II. GC analytical behavior of new compound

A. Dissolve reference standard material in acetone or iso-octane, pesticide-grade, to prepare stock solutions. Make all dilutions of the stock solution with acetone.

B. Determine amount (ng) of new compound which causes 50% FSD. Do not exceed 1000 ng injected.

C. Calculate RRc of new compound for all GC column/detector systems used.

III. Recovery of new compound through complete method

A. Select a representative nonfatty food sample. Analyze food by this method to assure that there are no residues in the sample which might interfere with the fortification test. Simultaneously, analyze a reagent blank for further information on the source of possible interferences.

Fortify duplicate (100 g) samples at 0.05-0.1 ppm. Analyze duplicate fortified samples as described in PAM I 232.4.

B. Fortify duplicate (100 g) samples at tolerance level for compound or, if no tolerance exists, at 5x level of (A). Analyze duplicate fortified samples.

IV. Report all results in a format which parallels this protocol. Include results of checks made on instrument parameters, as described in item I.C.

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Protocol IV

Background

Method: PAM I 242.2 and JAOAC (1980) 63 1114-1124

Compound types: Applicable to a variety of compound types; detection is dependent on availability of the particular detection step required for specific compounds, i.e.,

N-methylcarbamates: HPLC and post-column fluorescence labeling, with fluorescence detection

Naturally fluorescent pesticides: HPLC and fluorescence detection

Volatile and thermally stable pesticides: PAM I GLC detection methods

PAM I Tables: Table 201-J (recoveries through several applications of complete method)

Tables 330 (GLC data: tables for each column/detector combination).

[Also see JAOAC (1980) 63 1114-1124 for retention times of compounds through HPLC column.]

Data Development

I. Instrumentation

A. HPLC

1. N-methylcarbamates: Set up HPLC with post-column fluorescence labeling and fluorescence detector, as described in JAOAC (1980) 63 1114-1124. Check for proper operation using carbofuran as described in the reference.

2. Fluorescent pesticides: Set up HPLC and fluorescence detector, as described in J. Chromatogr. (1983) 255 497-510 and JAOAC (1983) 66 234-240. Check for proper operation using carbofuran as described in the references.

B. GLC. PAM Vol. I Chapter 3. If GLC is investigated, at a mimimum, OV-101 and DEGS liquid phases should be examined at standard conditions and operating para-

meters. Use chlorpyrifos as the reference compound. Use appropriate selective GLC detectors at standard operating parameters. (See Protocols I, II, and III for details on GLC operations.)

II. Instrument analytical behavior of new compound. (These directions refer to the two HPLC operations described above. See Protocols I, II, and III for details on collecting GLC data.)

A. Dissolve reference standard material in methanol to prepare stock solutions. Dilute with methanol for HPLC working standards. [Note: For GLC working standards, dilute stock solutions with whatever solvent is appropriate for the GLC system.]

B. Determine amount (ng) of compound which causes the detector to produce a 50% FSD recorder response. Note the peak shape of the compound to determine adequacy of chromatography.

C. Determine linear response range of the detector to the compound.

D. Determine stability of compound in methanol:

Short term stability study: Inject quantity of compound which produces 1/2 FSD response; repeat every 40 minutes for 8 hours.

Long term stability study: Inject quantity of compound which produces 1/2 FSD response; repeat once every day for eight days. Normalize response to carbofuran reference standard injected each day.

E. Calculate the retention time of the compound relative to carbofuran on the HPLC system of JAOAC (1980) 63 1114-1124 or JAOAC (1983) 66 234-240, as appropriate.

III. Recovery of new compound through cleanup column:

Initially determine that charcoal-silanized Celite column has proper elution characteristics as per carbamate method (JAOAC (1980) 63, 1114-1124). Pass 25 ug of pesticide through newly prepared column as per carbamate method. After collection of eluant (20 mL methylene chloride + 125 mL toluene-acetonitrile) in round bottom flask, momentarily stop flow, remove bottom flask and replace with a second round bottom flask. Elute column with an additional 100 ml toluene-acetonitrile. Evaporate solvents in both round bottom flasks to dryness as per method. Dissolve residue in first flask to appropriate volume with appropriate solvent for determination. Dissolve residue in 2nd flask with 5 mL of solvent and determine percent of total added

pesticide carried over into second flask. Continue recovery studies with food products, IV, only if combined recoveries from charcoal column are greater than 50%. If 10% or more of pesticide elutes in 2nd flask, collect a separate additional 100 mL eluting solution in recovery studies with food products.

IV. Recovery of new compound through complete method

A. Select a representative food sample. (This method has been found applicable to both fatty and nonfatty food.) Analyze food by this method to assure that there are no interferences. Simultaneously, analyze a reagent blank for further information on the source of possible interferences.

Fortify 150 g of chopped food product, while it is in homogenizer, at appropriately 0.05 ppm. Analyze duplicate fortified samples.

B. Fortify 150 g samples at a level near the tolerance or at 5 X the level in A if there is no tolerance. Analyze duplicate fortified samples.

V. Report all results in a format which parallels this protocol. Include the results obtained with reference compounds to establish appropriate instrument parameters, as described in items I. A. and B.

Division of Chemical Technology
Food and Drug Administration
September 1984

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